ISOAGGLUTINATION IN MAN AND LOWER ANIMALS.*†

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It is of interest that the normal serum of one individual may agglutinate the red blood cells of another of the same species, a phenomenon which Ehrlich named isoagglutination.

Landsteiner[1] first suggested that individuals might be divided into three distinct groups according to this phenomenon:

Group 1.—The corpuscles are not agglutinated by sera of the other two groups, while the sera agglutinate the corpuscles of both groups.

Group 2.—The corpuscles are agglutinated by the sera of the other two groups while the sera agglutinate the corpuscles of group 3, but not of group 1.

Group 3.—The corpuscles are agglutinated by the other two sera, and the sera agglutinate the corpuscles of group 2, but not of group 1.

Jansky[2] and Moss[3] divided individuals into four groups. Moss found isoagglutination in 90 per cent of the individuals tested. His grouping and the percentage in each group are as follows:

Group 1.—Ten per cent; serum agglutinates corpuscles of no group; corpuscles agglutinated by serum of groups 2, 3, and 4.

Group 2.—Forty per cent; serum agglutinates corpuscles of groups 1 and 3; corpuscles agglutinated by serum of groups 3 and 4.

Group 3.—Seven per cent; serum agglutinates corpuscles of groups 1 and 2; corpuscles agglutinated by serum of groups 2 and 4.

Group 4.—Forty-three per cent; serum agglutinates corpuscles of groups 1, 2, and 3; corpuscles are not agglutinated by any serum.

There seem to be no autoagglutinins.

Isoagglutination may possibly bring about disastrous results in transfusion of blood, and the selection of a donor should be made with reference to the isoagglutinative grouping. Manifestly the greatest danger lies in transfusion between members of groups 2 and 3. Cases with seemingly unfavorable results under these

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[1] Landsteiner, *Centralbl. f. Bakt.*, I, Orig., 1900, 27, p. 361; *Wien. klin. Wchnschr.*, 1901, 14, p. 1132; Landsteiner and Leiner, *Centralbl. f. Bakt.*, I, Orig., 1905, 38, p. 548; Landsteiner and Reich, *Ibid.*, 1905, 39, p. 712.

[2] Cited by Moss.

[3] *Johns Hopkins Hosp. Bull.*, 1910, 21, p. 63.

conditions have been reported by Schultz,[1] Ottenberg,[2] and Hopkins.[3]

Concerning the nature of the so-called isoagglutinins it has been shown that they are relatively thermostable, and resist heating to 55° C. for 30 minutes. Halban has stated that fetal blood from the cord at birth often contains isoagglutinin, even in cases in which the maternal blood did not contain isoagglutinins. Ottenberg and Epstein[4] and later von Dungern and Hirschfeld conclude that with respect to inheritance isoagglutination appears to follow the Mendelian law. When strong serum is used agglutination may take place almost instantaneously. The isoagglutinative substances are absorbed by the cells which they agglutinate. Hektoen[5] and Moss[6] found that the isoagglutination occurs with approximately the same relative frequency in health as in disease. Hektoen found that the concentration of the isoagglutinins is practically constant from day to day in the same individuals. Gay[7] urged that there is evidence for the belief that isoagglutination of human blood may be due simply to physico-chemical variation of molecular concentration and not dependent on the presence of any agglutinins.

In performing isoagglutinative tests various methods have been used. Practically all observers have used a five per cent suspension of corpuscles and mixed with a small amount of this suspension in small tubes an equal quantity of serum. Ottenberg and Epstein make the tests in capillary pipettes. In making tests on large groups, difficulty was experienced with both methods and a new method[8] was therefore attempted, which proved of great value in carrying out tests for isoagglutination rapidly in large groups.

Three drops of the blood are added to 10 c.c. of a 1 per cent solution of sodium citrate in physiologic salt solution. In this manner approximately a 2 per cent suspension of the blood is prepared, the citrate preventing coagulation. More blood is collected in a centrifuge tube and allowed to clot. With a clean needle the clot is loosened from the side of the tube and the tube centrifuged for a few minutes to obtain an upper layer of absolutely clear serum. This completes the preparation

[1] *Berl. klin. Wchnschr.*, 1910, 47, p. 1407.
[2] *Jour. Exper. Med.*, 1911, 13, p. 425.
[3] *Arch. Int. Med.*, 1910, 6, p. 270.
[4] *Trans. New York Path. Soc.*, 1908, 8, p. 117.
[5] *Jour. Infect. Dis.*, 1907, 4, p. 297.
[6] *Trans. Amer. Assoc. Physicians*, 1909, 24, p. 419.
[7] *Jour. Med. Res.*, 1908, 17, p. 321.
[8] *Jour. Amer. Med. Assoc.*, 1912, 59, p. 793.

of material, with the exception of the plate which is now described. On an ordinary piece of window glass, approximately $3\frac{1}{2}\times 9$ inches in size, which has been washed clean with water and ether, small circles are made with melted paraffin. If the melted paraffin is drawn up into a medicine dropper, the circles are quickly made with the tip while gentle pressure is exerted on the bulb. Ten circles may be made with one medicine dropper full of melted paraffin. In this manner paraffin cups are made, each of which will hold at least four drops. Assuming that a series of 20 are to be studied, 40 circles may be made on one such plate, four rows of 10 each. Ten such plates are prepared. Then into each cup of set No. 1 are placed two drops of serum 1; in each cup of set No. 2 are placed two drops of serum 2, etc. In each of the 20 cups in set No. 1 are placed next two drops of corpuscles No. 1, etc. If there is still likelihood of clotting, a drop of citrate solution may be added to each mixture. With a narrow glass rod the fluids are mixed thoroughly, the rod being washed in citrate solution and wiped after each mixing. In practically all instances, isoagglutination when present becomes visible macroscopically after half an hour at room temperature; the mixture can be inspected easily under the microscope also.

Two groups of normal individuals were examined and the same definite grouping as has been observed by the various investigators was found. A typical table is given (Table 1).

TABLE 1.

ISOAGGLUTINATION IN MAN.

Corpuscles	Serum																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.	o	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II.	o	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
III	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
IV	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
V	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
VI	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
VII	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
VIII	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
IX	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
X	o	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+
XI	o	o	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+
XII	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIII	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIV	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XV	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVI	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVII	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVIII	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIX	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XX	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

As isoagglutination would seem to be a phenomenon of biological importance, a study of the occurrence in various species was undertaken. Hektoen failed to observe isoagglutinins in the serum of rabbits, guinea-pigs, dogs, horses, and cattle, in each instance

using the serum and corpuscles of 10 to 20 different animals. Ottenberg and Friedman,[1] however, found that it did occur in rabbits and in steers. In rabbits it was found that there occurred a distinct grouping which bore no relation to race and color. These groups were as follows:

Group 1.—Serum agglutinative toward all cells, cells not agglutinable.
Group 2.—Serum agglutinates group 3 cells, cells agglutinated by serum 1.
Group 3.—Serum non-agglutinative, cells agglutinated by sera 1 and 2.
Group 4.—Serum non-agglutinative, cells not agglutinable.

In steers it was found that a grouping could be made into three sets:

Group 1.—Agglutinative but not agglutinable.
Group 2.—Agglutinable but not agglutinative.
Group 3.—Neither agglutinative nor agglutinable.

More recently Ingebrigsten[2] studied the occurrence of isoagglutination in 40 cats. He found in at least five cases positive interagglutination. In other instances there was some doubt. In 32 no agglutination was present. No grouping was possible.

I have examined several different species including 60 swine, 60 cattle, 40 sheep, 25 rabbits, 20 frogs, and 10 dogs. Tables 2, 3, 4, and 5 of groups of twenty are typical of the results.

TABLE 2.

ISOAGGLUTINATION IN SHEEP.

Corpuscles	Serum																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
II.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
III.....	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
IV.....	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
V.....	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
VI.....	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
VII.....	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
VIII....	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
IX.....	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
X.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XI.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XII.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIII....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIV.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XV.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVI.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVII....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVIII...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIX.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XX.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

[1] *Jour. Exper. Med.*, 1911, 13, p. 531.

[2] *München. med. Wchnschr.*, 1912, 59, p. 1475.

In studying isoagglutination in these animals, an endeavor was made to determine whether race or color played any part. It was found that the occurrence of agglutination did not bear any relationship to these factors.

It was found further that isoagglutination in animals is a much less pronounced phenomenon than in man. In many

TABLE 3.

ISOAGGLUTINATION IN SWINE.

Corpuscles	Serum																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.	o	o	o	o	o	o	o	o	o	+	o	+	+	+	+	o	o	o	+	o
II.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o
III.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
IV.	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o
V.	+	o	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o
VI.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
VII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o
VIII.	+	o	o	o	o	o	o	o	o	o	o	o	+	o	+	+	+	o	+	o
IX.	o	o	o	o	+	o	o	o	o	o	+	o	o	o	+	o	o	o	+	o
X.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XI.	o	o	o	o	o	+	o	o	o	o	o	+	o	o	o	o	o	o	o	o
XII.	+	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o
XIII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o
XIV.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XV.	+	o	o	+	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o
XVI.	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o	o	o	o
XVII.	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o
XVIII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIX.	+	o	o	o	+	o	o	+	+	+	+	+	o	+	+	+	o	o	o	+
XX.	+	+	+	o	+	o	o	o	o	o	o	o	o	o	o	+	o	o	+	o

TABLE 4.

ISOAGGLUTINATION IN CATTLE.

Corpuscles	Serum																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
II.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	+	o	+	o	o
III.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
IV.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
V.	+	+	o	o	o	o	o	o	o	o	o	o	o	+	o	+	o	o	o	o
VI.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o
VII.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
VIII.	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
IX.	+	+	+	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	+	+
X.	o	+	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XI.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o
XII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIII.	o	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIV.	+	+	o	o	+	o	o	o	o	o	o	o	o	o	+	o	+	+	+	o
XV.	o	+	o	o	o	o	o	o	+	o	o	o	o	o	o	o	o	o	o	o
XVI.	o	o	+	o	o	o	o	o	o	o	o	o	o	o	+	o	+	o	o	o
XVII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XVIII.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XIX.	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
XX.	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o	o	o	o	o	o

TABLE 5.

ISOAGGLUTINATION IN RABBITS.

Corpuscles	Serum.																				
	1	2	3	4	5	6	6	8	9	10	11	12	13	14	15	16	17	18	19	20	Species
I.....	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Tan
II.....	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o	+	o	o	o	o	Grey White
III.....	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o	+	o	o	o	o	Belgian
IV.....	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o	+	o	o	o	o	"
V.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	"
VI.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	"
VII....	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o	"
VIII...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Black
IX.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Grey White
X.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Tan
XI.....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Grey White
XII....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Grey and White
XIII...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Grey and White
XIV...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	Black
XV....	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	Maltese
XVI...	o	o	o	o	o	o	o	o	o	o	+	o	o	+	o	o	o	o	o	o	"
XVII...	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o	White
XVIII..	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	o	o	o	o	o	"
XIX...	o	o	o	o	o	o	o	o	o	o	o	o	o	+	o	+	o	o	o	o	"
XX....	o	o	o	+	o	o	o	o	o	o	o	o	o	+	o	+	o	o	o	o	"

instances, only by microscopical examination could it be accurately determined whether agglutination had occurred or not. This was particularly true in the case of frogs and rabbits.

It is obvious from the tables that no division into groups can be consistently made. Just as Ingebrigsten found to be the case in cats, so in dogs, sheep, cattle, swine, and rabbits, isoagglutination occurs but seems to be governed by no definite grouping. In all instances there is indeed a large group which is neither agglutinative nor agglutinable, this being particularly true of sheep and rabbits, but beyond this nothing further can be said.

In the frogs examined, isoagglutination did not occur. All specimens were examined microscopically at various intervals after mixing, but not the slightest trace of agglutination was visible.

With regard to isoagglutination in swine, Reichel[1] states that in intravenous injection of hogs with hog serum, deaths have been observed with all the evidences of intravascular agglutination.

The mechanism of this phenomenon is still in doubt. Various interesting hypotheses have been advanced and but few experiments made in their support. The assumption is usually made of

[1] Personal communication.

various agglutinins and agglutinophilic receptors. Thus Moss advances the following explanation for the grouping in man:

Group 1.—Serum contains no agglutinin, corpuscles possess receptors *a*, *b*, and *c*.
Group 2.—Serum contains agglutinin A, corpuscles possess receptors *b* and *c*.
Group 3.—Serum contains agglutinin B, corpuscles possess receptors *a* and *c*.
Group 4.—Serum contains agglutinin C, corpuscles possess no receptors.

It would seem that the only other possibility would be the reverse, agglutinins being substituted for receptors and vice versa. The first hypothesis is proved, says Moss, by using the serum of group 4 to agglutinate the corpuscles of group 1, after which it would not agglutinate corpuscles of groups 2 and 3.

In discussing their conclusions as to grouping in rabbits and steers, Ottenberg and Friedman also hypothecate agglutinins and agglutinable substances. The grouping in the rabbits calls for two of each and in the steers for one of each.

My results show in all cases that agglutination seems to have occurred without any definite grouping. To account for each individual instance in the case of the swine, let us say, according to such a hypothesis, would demand a host of such receptors and agglutinins. In man, isoagglutination is distinctly a group reaction; it is constant, and it is accompanied definitely by absorption of some substances which seem to govern the reaction, so that it would seem to be due to something else than physico-chemical variations of molecular concentration. In the case of animals, rather than assume the presence of multitudinous agglutinins and receptors, it would be simpler to assume the existence of one agglutinin and one receptor only, whose actions are modified by extraneous factors, as, for instance, chemical and physical variations, perhaps infinitesimal, in the individual sera and corpuscles tested.

In conclusion, then, it appears that in man there is a distinct isoagglutinative grouping possible; that in other mammals, isoagglutination is present but according to no special order, and that in frogs, as representing amphibians, it appears to be absent. It is suggested that where facilities permit, tests be made on a wide variety of species, including monkeys and higher apes, to see whether any definite gradation exists.